Evaluating Strategies for the Treatment of Cerebral Cavernous Malformations

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Abstract—Cerebral cavernous malformations are common vascular lesions of the central nervous system that predispose to seizures, focal neurological deficits, and potentially fatal hemorrhagic stroke. Human genetic studies have identified 3 genes associated with the disease, and biochemical studies of these proteins have identified interaction partners and possible signaling pathways. A recurring theme dominating the recent scientific literature is the causal link between mutations in the 3 *cerebral cavernous malformation* genes and hyperactivation of the small GTP exchange protein, RhoA, and the efficacy of reducing this hyperactivation using inexpensive and well-studied medicines, statins. Familial cerebral cavernous malformation offers a unique opportunity to use a personalized genomic medicine approach to identify a subset of patients prone to intracerebral hemorrhage that may benefit from a pharmacological therapy, where presently only neurosurgical options are available. (*Stroke*. 2010;41[suppl 1]:S92-S94.)

Key Words: intracerebral hemorrhage ■ treatment ■ cerebral cavernous malformation

There are many recent reviews on the genetic basis, animal models, and biochemical signaling associated with cerebral cavernous malformations (CCMs).[1–3] In distinction to those in-depth reviews, we focus on assessing whether sufficient scientific progress and consensus have been achieved to warrant clinical trials that examine a specific mechanistic hypothesis: statin treatment may improve the outcome of CCMs. We need to emphasize that the point of view of this article is heavily influenced by past and current clinical trials examining the role of statins in the much broader population of intracerebral hemorrhage (ICH) patients and the growing support for genomic medicine approaches that advocate identifying subset of patients who are most likely to benefit from any proposed therapy.

CCMs are common vascular malformations described predominantly in the central nervous system. Lesions consist of dilated endothelial channels or caverns lacking smooth muscle support and filled with blood or thrombus. Occasionally CCMs rupture leading to hemorrhagic stroke or death. Even in the absence of overt hemorrhage, all lesions are associated with hemosiderin, a blood breakdown product in the surrounding brain parenchyma. Ultrastructural studies suggest absent or diminished tight junctions, implying localized loss of the blood–brain barrier and loss of vascular stability.

CCM lesions are found with a prevalence of 1 in 200 people. Approximately 20% of patients have familial disease, following an autosomal dominant pattern of inheritance. CCM has been linked to loss-of-function mutations in the genes encoding any of 3 structurally distinct proteins, *KRIT1*

(aka C*CM1*), *CCM2* (aka *Osmosensing Scaffold for MEKK3 – OSM*, *Malcavernin*, or MGC4607), or *Programmed cell death 10* (*PDCD10*, aka *CCM3*). The 3 CCM disease genes are structurally unrelated intracellular proteins that lack catalytic domains. Driven by their common link to disease, experiments were performed that found these 3 gene products may associate with one another to influence a variety of signaling pathways. For 2 of the genes, *KRIT1* (*CCM1*) and *OSM* (*CCM2*) there is consistent biochemical, cell biology, and animal model data to support a role for RhoA hyperactivation and endothelial instability after loss of function mutations. Importantly, reduction of RhoA hyperactivation with drugs, statins, and fasudil ameliorated the pathobiology caused by loss-of-function mutations in these 2 genes.

Initial evidence that CCM might result from abnormal GTPase activity came with the identification of *KRIT1* mutations in families with CCM. Further proof that the KRIT1–RAP1a interaction is functionally important came from studies in endothelial cells in vitro[4] and *Krit1* heterozygous knockout mice in vivo.[5] KRIT1 and RAP1a interact to influence endothelial cell junctional integrity through downstream signaling cascades such as β-catenin signaling. KRIT1 has also been shown to interact genetically with the related GTPase, RAP1b.[6]

CCM2 was identified simultaneously as a gene associated with familial CCM and as a scaffold protein to facilitate stress signaling from the small GTPase Rac1 to p38 MAPK.[7] CCM2 also associates with RhoA, and the loss of CCM2 leads to increased RhoA activity in the endothelium.[8] In-

creased RhoA activity is associated with vascular instability, and favorable effects of RhoA inhibition by statin therapy in *CCM2* deficiency are seen both in vitro and in vivo, suggesting a possible therapeutic strategy for CCM disease.[8] Statins inhibit HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis. Inhibition of HMG-COA reductase not only reduces cholesterol production, it also reduces gerranylgeranyl-diphosphate, a lipid necessary for the isoprenylation of RhoA, a process critical for tethering RhoA to the cell membrane making it available for activation. Multiple laboratory groups highlight the critical role of RhoA hyperactivation in CCM biology. Stockton and colleagues showed that mutations in 2 of the *CCM* genes, *KRIT1* (*CCM1*) and *OSM* (*CCM2*), resulted in Rho hyperactivation, and direct inhibition with a ROCK inhibitor, fasudil, was effective in cell culture and in animal models.[9] Borikova and colleagues report dramatic increases in RhoA after loss of function mutations in each of the 3 *CCM* genes, and demonstrate that an shRNA approach effectively reverses endothelial defects caused by *CCM* deficiency.[10]

Loss-of-function mutations in *CCM1* or *CCM2* are the predominant genetic causes for CCMs.[11,12] In both cases, mice and endothelial cells lacking these 2 genes phenocopy one another, and there is experimental evidence that reversing RhoA activation in culture and in animal models reduces the pathobiology. There is experimental evidence that KRIT 1 (CCM1) and OSM (CCM2) proteins directly bind to one another, suggesting that the 2 participate in a common biochemical mechanism.[13] The evidence for a role of *PDCD10* (*CCM3*) in a similar pathway is not as strong as for the other 2 CCM genes. One report suggests evidence for Rho activation, but work by Wang Min focuses on a distinct role for *PDCD10* in VEGF signaling, and at present there is no animal studies to suggest that reducing RhoA activation has a beneficial effect in vivo.[13–15] In summary, there is a consensus from multiple laboratories using different approaches that span biochemistry, cell biology, and animal models that loss of function mutations in 2 *CCM* (*KRIT1* and *OSM*) genes cause RhoA activation, and that blocking this hyperactivation may strike at the mechanistic heart of disease pathogenesis.

It would be remiss not to highlight the considerable holes in our knowledge concerning CCM biology and disease. Mechanistically, we are at an infancy stage of understanding the signaling cascades controlled by *CCM* genes. The identity of upstream signals that control CCM mediated pathways,[16] the biochemical details of the direct protein interactions of *CCM* genes with one another as well as downstream effector molecules, and the predilection of human *CCM* mutations to manifest in the central nervous system vasculature are fertile grounds for scientific investigation. However, in terms of proposing clinical interventions, the most striking deficiency is the fact that none of the animal models generated by gene targeted mice phenocopy the human disease.

It must be recognized that most mice models that genocopy a human disease fail to phenocopy the human disease. Mice lacking 2 alleles of *CCM* genes fail to survive embryogenesis.[8,17,18] Mice lacking 1 allele of a *CCM* gene genocopies the human disease, yet these mice lack any evidence of vascular malformations in the central nervous system. These mice are susceptible to environmental stress in the form of cytokines, resulting in pathological vascular leakage, and this excessive leakage can be blocked by drugs such as fasudil or statins.[8,9] However, one cannot extrapolate these findings into a demonstration that such pharmacological interventions successfully reduced CCM progression or severity in animal models. There are many logical avenues for developing a more faithful animal model of CCM. They include the possibility that the human disease CCM may represent a 2-hit model: the first hit being a germline mutation in a CCM allele; the second hit can either take the form of a somatic second hit in the other CCM allele or in the form of inflammatory stress that reveals the susceptibility of the central nervous system endothelium. There is limited evidence in clinical data to support such mechanistic hypotheses, so experimental evidence using animal models will contribute greatly to examining whether such mechanisms are even plausible.[19,20]

Success in reproducing human disease in animal models will not only test these key scientific hypotheses concerning disease pathogenesis, it will make it possible to test therapeutic strategies in animal models using therapies and end points analogous to any future clinical trial. One might argue that the generation of a faithful animal model for CCM is a litmus test for examining the mechanistic hypothesis that reduction of RhoA hyperactivation by statins or other drugs will be effective in reducing cerebral vascular malformations caused by loss-of-function mutations in CCM genes. From a scientific viewpoint, such an achievement would open an idealized approach for translating scientific and mechanistic insights into clinical practice. There is a precedent for such a strategy in the seminal work of Hal Dietz and his collaborators with respect to Marfans syndrome.[21–24] These investigators developed mechanistic insights and faithful animal models that allowed them to test the efficacy of angiotensin receptor blockers in reducing aortic dilation. These scientific findings triggered a nonrandomized clinical study, which, in turn, has led to larger randomized trials.[25] As scientific teams race to adopt a similar approach for CCM, it must be recognized that faithful animal models, even with the advent of mammalian gene targeting, are extremely difficult to achieve. Some notable examples include the lack of significant coronary artery disease in murine models of familial hypercholesterolemia or the lack of prominent pulmonary complications in mice models of cystic fibrosis. Gene-targeting strategies produce models that provide valuable understanding of the developmental function of the targeted gene in vivo. In the context of genes associated with human disease, they have uncovered fundamental insights into the gene's function. The lack of fidelity of animal models in reproducing human disease phenotypes does not diminish the power and importance of this tool, but it throws caution on a rigid strategy based on demanding evidence of successful therapeutic intervention in a faithful genetic animal model that phenocopies the human disease before advancing such therapies into the clinic.

We have outlined the idealized goal of translating understanding into the biology and pathogenesis of CCM to the treatment of clinical CCM. An alternative strategy is to proceed with investigating the efficacy of statins in the CCM

clinical population. There are many components of such a trial that will need to be considered including whether to restrict testing to patients with CCM1 or CCM2, the 2 genotypes with the strongest evidence in support of statin therapy, and the specific end points and patient recruitment needed for such clinical trials. However, the crux lies in establishing whether it is reasonable to initiate clinical studies for statin use in CCM patients. It should be noted that the field has decided that trials examining whether statins improve the outcome of the broader ICH population may be warranted given competing evidence for their efficacy.[26–29] Though there is some evidence that statins may be beneficial in animal models of ischemic stroke and ICH,[30,31] the body of evidence that mechanistically links CCM biology and disease with statins is stronger and more direct than for the general ICH population. In fact, there is considerable interest in all diseases including ICH to define subgroups of patients by genome wide association or other genomic technologies that are most likely to benefit from a given therapeutic intervention. In the case of CCM, the genetic association of patient to disease is much stronger than these other strategies. Thus, if investigational studies investigating the efficacy of statins for the ICH population are justified, then an even stronger argument can be made for investigating the impact of statin use in patients with CCM, a disease with a direct mechanistic link to the proposed intervention.

Disclosures

None.

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